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Acanthostral, a Novel Antineoplastic cis, cis, cis-Germacranolide from *Acanthospermum australe*

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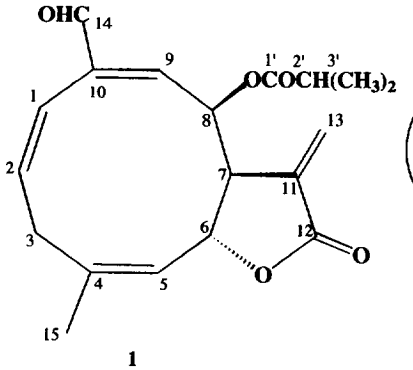
Abstract: Acanthostral (**1**), a novel germacranolide, was isolated as an antineoplastic constituent of *A. australe*, and its structure was elucidated by spectroscopic methods.

In our screening program for biologically active substances from medicinal plants, we have devoted our attention to the occurrence of natural products having an anticancer activity. In this paper, we describe the isolation and structure determination of acanthostral (**1**), a novel antineoplastic germacranolide from the Paraguayan plant "Tapecue", *Acanthospermum australe* O.K[1]. "Tapecue" is an important, traditional crude drug used widely in South America. From this plant, many melampolides, a subgroup of germacranolide, and flavonoids have been isolated[2-6]. Some of them have been characterized by antineoplastic activity[7].

The above-ground plants (2 kg) of *A. australe* were extracted with 10 l of benzene. The benzene extract was directly subjected to repeated chromatographies over silica gel with the solvent system (hexane-EtOAc; 100:1, 50:1, 20:1, 10:1, 5:1, 1:1) to give the active fraction. The fraction was separated by silica gel TLC (solvent: hexane-EtOAc; 2 : 1), and purified by HPLC (Develosil ODS HG-5, Nomura Chemical, 8x250 mm; flow rate, 1.0 ml/min; uv detection at 254 nm; eluant, 90 % MeCN) to give acanthostral (**1**)[8] (23 mg) as a colorless oil. The separation was monitored by inhibitory activity against L1210 cell culture.

Acanthostral (**1**) showed a molecular ion at *m/z* 330 in the EIMS. The IR spectrum of **1** displayed absorption bands at 1770, 1744 and 1697 cm^{-1}, indicating the presence of an α,β-unsaturated lactone, a saturated ester and an α,β-unsaturated aldehyde, respectively. No hydroxy absorption was observed. The ^1H NMR spectrum confirmed the presence of an α-methylene lactone with doublet signals at δ 6.39 (J = 3.3 Hz) and δ 6.14 (J = 2.9 Hz) for H-13a and H-13b, respectively. All the ^1H NMR signals were firmly assigned by the ^1H-^1H COSY spectrum. Multiplicities of the ^{13}C signals were determined by DEPT experiments. The assignment of the carbons bearing hydrogens was established by ^{13}C-^1H COSY via one-bond coupling. Long-range couplings (two- and three-bonds) were detected in the COLOC spectrum which established assignment of the carbons without hydrogen. The configurations of the three double bonds were elucidated as follows. The H-1 proton appeared as a doublet with the coupling constant of 11.4 Hz, indicating that the 1,2-double bond was typically cis geometry. In the ^{13}C NMR, the chemical shift value of the vinyl methyl at C-15 appeared at δ 23.7. This indicated that the 4,5-double bond was cis geometry. The 9,10-double bond was also determined as cis, because in the difference NOE experiment, irradiation at the frequency of the vinyl proton at H-9 (δ 6.54) increased 24% in the intensity of the aldehyde proton at H-14 (δ 9.54). The problem then centered on the stereochemistry. The configuration of the 7,11-bond in all well characterized sesquiterpene lactones is β, and from Samek's rule[9] the value of the coupling constant (3.3 and 2.9 Hz) of H-7 with H-13 exo-methylene protons indicated a trans-fused lactone, placing the H-6 in a β-stereochemistry. This was confirmed by the NOE experiment. Irradiation at the frequency of H-7 (δ 3.21) enhanced 9% in the intensity of the vinyl proton at H-5 (δ 5.26). The configuration of the H-8 proton was established in α-orientation according to the NOE measurement. Irradiation at the frequency of H-7 (δ 3.21) produced 15% enhancement in the intensity of H-8 (δ 5.73). The conformation of ten-membered

ring in **1** was elucidated by the NOE data (Fig. 1) and coupling constants (H-2/H-3a, 12.3 Hz; H-2/H-3b, 5.5 Hz; H-8/H-9, 10.3 Hz). Finally, the structure of **1** was determined as shown.



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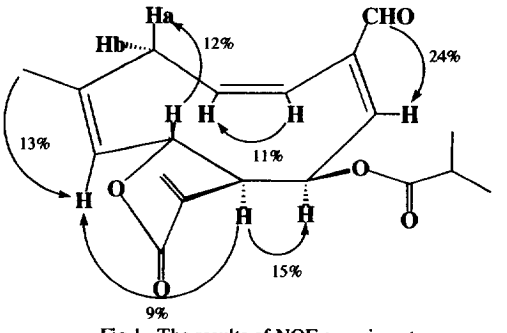

Fig. 1. The results of NOE experiment

Acanthostral (**1**) showed significant antineoplastic activity against L1210 cell culture with ED_{50} value of $5x10^{-6}$ M, but had no effect against the enzyme activities of cyclic AMP phosphodiesterase, Na^+-K^+ ATPase and Ca^{2+} ATPase, or the function of sarcoplasmic reticulum. Any other physiological functions of this class of compounds would be of interest for future investigation.

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8. **1**: $[\alpha]_D^{20}$ -30° (c = 2.0, MeOH), UV: λmax(MeOH) 214 (ε 6515) nm, EIMS *m/z* $[M]^+$ 330, 301, 260, 71; HRMS: found 330.1465, calcd. 330.1467 for $C_{19}H_{22}O_5$; IR(film) 1770, 1744 and 1697cm^{-1}; ^{1}H NMR(500 MHz, $CDCl_3$):δ9.54(1H, d, J =1.5 Hz, H-14), 6.54(1H, dd,J = 10.3, 1.5 Hz, H-9), 6.39(1H, d, J = 3.3 Hz, H-13a), 6.14(1H, d, J = 2.9 Hz, H-13b), 6.11(1H, m, H-2), 5.94(1H, d, J = 11.4 Hz, H-1), 5.73(1H, dd, J = 10.3, 5.1 Hz, H-8), 5.26(1H, d, J = 9.5 Hz, H-5), 4.87(1H, t, J = 9.5 Hz, H-6), 3.21(1H, m, H-7), 2.97(1H, t, J = 12.3 Hz, H-3), 2.57(1H, qq, J = 7.0 Hz, H-2'), 2.39(1H, dd, J = 12.3, 5.5 Hz, H-3), 1.86(3H, br s, H-15), 1.15(6H, d, J = 7.0 Hz, H-3'); ^{13}C NMR(125MHz, $CDCl_3$): δ193(C-14), 175(C-1'), 169(C-12), 146(C-10), 143(C-9), 142(C-4), 135(C-11), 133(C-2), 123(C-13), 122(C-1), 121(C-5), 75.8(C-6), 68.9(C-8), 47.6(C-7), 34.0(C-2'), 33.7(C-3), 23.7(C-15), 11.5(C-3').
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